

Amendments to the Specification

Please replace the BRIEF DESCRIPTION OF THE DRAWINGS section with the following amended BRIEF DESCRIPTION OF THE DRAWINGS:

BRIEF DESCRIPTION OF THE DRAWINGS

~~FIG. 1. (a) Experimental device. Left: the device with the channels filled with bromthymol blue in 10 mM NaOH solution for visualization. Right: different ratios of the widths of the aqueous:organic phase, a-1:3, b-1:1, c-3:1.~~

~~FIG. 2~~ FIG. 1. Model computations. Efficiency of ion exchange for liquid ion exchanger and suspended ion exchange resin particles of different diameter.

~~FIG. 3. FIGS. 2A – 2D.~~ Model computations. The effect of variation of system parameters on the fraction of the eluent exchanged. See text for details.

~~FIG. 4~~ FIG. 3. The effect of (a) the width and (b) the flow rate of the eluent flow stream on ion exchange efficiency. Conditions: 10 mM HCl eluent, ion exchanger 150 mM TOAOH in butanol+1% Triton X-100. (a): flow rate  $5 \mu\text{L min}^{-1}$  (i) nonsuppressed eluent, (ii) eluent width 750  $\mu\text{m}$  (iii) eluent width 500  $\mu\text{m}$ , (iv) eluent width 250  $\mu\text{m}$ ; (b): eluent channel width 500  $\mu\text{m}$ , (i) nonsuppressed eluent, (ii) flow rate  $10 \mu\text{L min}^{-1}$ , (iii) flow rate  $5 \mu\text{L min}^{-1}$ , (iv) flow rate  $2 \mu\text{L min}^{-1}$ . Note that the conductivity detector output is not completely linear with the actual specific conductance over the entire region, as such we have provided individual ordinate markings in this and similar figures rather than providing a linearly calibrated axis.

~~FIG. 5~~ FIG. 4. Comparison of suppressed conductance values obtained with different eluent-ion exchanger combinations.

~~FIG. 6~~ FIG. 5. Triplicate injections of 0.5 mM NaCl into an 1 mM HCl eluent phase. Conditions: injection volume: 650 nL, flow rate:  $2 \mu\text{L min}^{-1}$ , eluent width 250  $\mu\text{m}$ , (a) 15 mM TOAOH in butanol, 1% Triton X-100; (b) 1% Amberlite LA-2 in butanol containing 1% Triton X-100.

**FIG. 7** FIG. 6. Comparison of suppressed conductance values obtained with different eluent-suspended ion exchanger combinations.

**FIG. 8** FIG. 7. Conductivity detection of various heavy metals in the system with 1% Amberlite LA-2 in butanol, 1% Triton X-100. Conditions are the same as those for FIG. 6b. 1 mM HNO<sub>3</sub> was used as eluent for silver nitrate injection.

**FIG. 9** FIG. 8. The scheme of the device for continuous extraction /ion exchange in vertically stratified horizontal flows (upper part). Lower part : Process of extraction (a), ion-pair extraction (b,c) and ion exchange (d).

**FIG. 10** FIG. 9. Simulations of horizontally layered flows: left-butanol/water, right-chloroform/water. Linear flow rates (a)-1 mm.s<sup>-1</sup>, (b) -5 mm.s<sup>-1</sup>, (c) – 10 mm.s<sup>-1</sup>, (d) - 15 mm.s<sup>-1</sup>, (e) - 20 mm.s<sup>-1</sup>, (f) - 30 mm.s<sup>-1</sup>.

**FIG. 11** FIGS. 10A – 10C. Simulation of the effect of selected physical properties of the liquids on the formation of horizontally layered flows in the butanol/water system. (a) –reversed gravity, (b) – the effect of various contact angles, (c) – increase in the interfacial surface tension.

**FIG. 12** FIG. 11. The effect of the flow rate (a) and channel depth (b) on the extraction efficiency of 1 mM methylene blue (MB). Conditions: (a) channel depth: 400 µm, (b) flow rate: 40 µL.min<sup>-1</sup>, absorbance of the collected and diluted solutions measured at 565 nm.

**FIG. 13** FIG. 12. Extraction of DBSA-MB ion pair into hexanol. (a) – calibration graph for 2-20 ppm DBSA, (b, c) – 12 repeated injections of 12 ppm and 6 ppm DBSA standard. Conditions: channel depth 400 µm, flow rate 40 µL.min<sup>-1</sup>, injection volume 5 µL, absorbance measured at 565 nm.

**FIG. 14** FIG. 13. Analysis of extractable matter in tap water. (a) 3 injections of deionized water, (b) 3 injections of tap water. Conditions the same as in figure 5.

**FIG. 15** FIG. 14. 12 repeated injections of 1 mM potassium chromate. Conditions: channel depth 400 µm, flow rate 40 µL.min<sup>-1</sup>, injection volume 5 µL, absorbance measured at 372 nm.

**FIG. 16 FIGS. 15A – 15B.** Ion exchange/suppression in two immiscible liquids. (a) 12 injections of 1 mM NaCl into 1 mM HCl. Ion exchanger: 1% Amberlite LA-2 in butanol. Conditions: channel depth 400  $\mu\text{m}$ , flow rate 20  $\mu\text{l}\cdot\text{min}^{-1}$ , injection volume 5  $\mu\text{L}$ , conductivity detection.

Please replace the identified paragraphs of the specification with amended paragraphs as follows:

Page 20, first paragraph (lines 1-12):

For a system with the eluent and exchanger phase each occupying a width of 500  $\mu\text{m}$ , a length of 5 cm, with 10 mM HCl as eluent (Cl diffusion coefficient 2000  $\mu\text{m}^2/\text{s}$ ) being exchanged with a hydroxide form anion exchanger, Figure [[2]] 1 shows the computed fraction exchanged as a function of flow rate for three different types of exchangers: (a) 1  $\mu\text{m}$  size ion exchange resin suspension, (b) 100 nm size ion exchange resin suspension, and (c) dissolved TOAOH, the equivalent exchanger concentration being 50 mM in each case. The diffusion coefficient of the particulate exchangers were estimated, assuming spherical shape and the Stokes-Einstein equation, to be 0.215 and 2.15  $\mu\text{m}^2/\text{s}$  for 1 and 0.1  $\mu\text{m}$  diameter particles, respectively. The diffusion coefficient of TOAOH was estimated from the equivalent conductance values of tetraalkylammonium ions ranging from tetramethyl- to tetrabutyl- and applying a model:

Page 20, second paragraph (lines 16-26):

Where  $G$  is the equivalent conductance of the tetraalkylammonium ion,  $n$  is the number of carbon atoms in the alkyl chain, and  $a$  and  $b$  are constants. The available data fits this model (linear correlation coefficient,  $r^2 > 0.99$ ) and leads to an extrapolated value of  $G$  for  $\text{TOA}^+$  of 11.1  $\text{S.cm}^2$  equivalent. The Nemst-Einstein equation then allows the estimation of the diffusion coefficient of TOAOH of  $\text{TOA}\text{Cl}$  (which are largely controlled by the size of the  $\text{TOA}^+$  moiety) as 294  $\mu\text{m}^2/\text{s}$ . The results in Figure [[2]] 1 show that the dissolved exchanger with its much greater diffusion coefficient is obviously more effective. However, it would be possible to use a more concentrated suspension of a particulate exchanger without risk of bleedoff and confine it to a narrower section of the total channel width to make more efficient use of the exchanger.

Page 20, last paragraph (lines 28-31):

In Figure [[3]] 2, we show other computational results for parametric variations. In Figure [[3a]] 2A, the width of the 5 cm long channel is varied while 10 mM HCl is used as eluent and 50 mM TOAOH is used as the exchanger, both flowing at 5  $\mu\text{L}/\text{min}$ . The greater efficiency of smaller channel widths is readily apparent.

Page 21, first paragraph (lines 1-13):

Figure [[3b]] 2B shows a case where the fraction exchanged is plotted against the channel length for the suppression of 40 mM HCl with 50 mM TOAOH flowing at 5  $\mu\text{m}/\text{min}$  in a device where each phase occupies a 100  $\mu\text{m}$  width. Sufficient length is necessary to make good utilization of the regenerant even in a narrow channel width. Figure [[3c]] 2C shows a case identical to that in Figure [[3b]] 2B except that the channel length is fixed at 5 cm and the eluent concentration varied. This resembles typical suppressor behavior, insomuch as suppression is quantitative below some maximum eluent concentration. Figure [[3d]] 2D has the other parameters set the same as Figures [[3b]] 2B and [[3c]] 2C except that the eluent concentration is fixed at 10 mM and the diffusion coefficient of the ion to be exchanged is varied. In cation chromatography, where either HCl or  $\text{HClO}_4$  (or any other acid) can be used as eluent, it is advantageous to use a small mobile counterion containing acid such as HCl.

Page 29, line 3:

**Table 2 Table 3. Properties of solutions for simulation of horizontally layered flows.**

Page 29, second paragraph (lines 19-29):

The volume-of-fluid (VOF) interface model was used to track the fluid interface. Full surface tension effects were activated, including both normal and tangential surface tension forces. Gravity was applied in the negative  $z$ -direction. Figure [[10]] 9 shows the results at various input velocities. As can be seen there, the layered configuration becomes stable for the butanol/water flow at somewhere between 5 and 10 mm/sec input velocities, while the chloroform/water flow always assumes the segmented configuration. Experimentally the butanol/water layered flow became stable between 1 and 5 mm/sec, while the chloroform/water layered flow could not be stabilized at any velocity. Thus we have a good qualitative prediction of the flow behavior, but the simulation accuracy does not seem sufficient for high precision modeling.

Page 29/30, the paragraph spanning lines 30-34 on page 29 through lines 1-9 on page 30:

Parameters relating to the stability of the flow pattern include density, interfacial surface tension and wetting angle. Investigations were performed using a 1 cm channel. Butanol is less dense than water, hence the layered configuration studied in which butanol flows on top should be stabilized by buoyancy effects. However flow experiments indicate that the butanol/water layered flow is also stable when

butanol enters on the bottom. To test this in simulation the direction of gravity was reversed. As can be seen from Fig. [[11a]] 10A this layered flow is stable. The “wettability” of the channel walls is characterized by the interior contact angle of a drop of liquid on the channel surface surrounded by water. A contact angle of 0 degrees indicates perfect wetting, while a contact angle of 180 degrees indicates that the liquid does not wet. Because the sidewalls of our channels are made of several different materials, namely electrical tape and transparency film, while the floor and ceiling are glass, several values were considered. These were 0, 30, 60, 90, 120, 150 and 180. Selected results are shown in Figure [[11b]] 10B.

Page 30, first full paragraph (lines 11-16):

To test the effect of surface tension we re-ran the 20 mm/sec butanol/water case, but with the value for interfacial surface tension changed from that of butanol to that of chloroform. The resulting flow segments, as is shown in Fig. [[11c]] 10C. Recall that the 20 mm/sec isobutanol/water layered flow is stable. Therefore the interfacial surface tension seems to be a major factor determining a stable flow configuration.

Page 30, last paragraph (lines 18-30):

**Continuous extraction.** To study the function and parameters of the microfluidic device with the horizontal flows a model extraction system was designed. A 1 mM solution of methylene blue (MB) in deionized water was aspirated through the device on the bottom of the channel and an immiscible solution of a short chain aliphatic alcohol on the top of the channel. The upper organic phase with the extracted MB was collected, diluted with respective solvent and its absorbance was measured at 656 nm. The results of varying the flow rates between 20 and 160  $\mu$ L/min is shown in figure 12a Figure 11A. The efficiency of the extraction increases with decreasing the flow rate, as expected from the Fick’s law of diffusion. A significant difference between the amount of MB extracted into different alcohols can be observed too. The low extraction efficiency of hexanol could be advantageously used for ion-pair extraction of MB and anionic surfactant (DBSA).

Page 31, first paragraph (lines 1-8):

On FIG. [[12]] 11, the effect of the channel depth on the extraction is shown. The same solutions as in FIG. [[12a]] 11A were used in three channels differing only in the depth. The volumetric flow rates were recalculated to the linear flow rates in mm/s to achieve the same contact time of the solutions in respective channels. In the design with the channel depth of 150  $\mu$ m, the thickness of the layered flows is

approximately 75  $\mu\text{m}$ , e.g. the diffusion path for the methylene blue is almost 3 times shorter than for the depth of 400  $\mu\text{m}$ . Clearly the decrease in channel depth favors the extraction..

Pages 31/32, the paragraph spanning page 31, lines 27-31 through page 32, lines 1-13:

The extraction efficiency of methylene blue into hexanol is rather poor as shown in FIG. [[12a]] 11A. The amount of MB extracted can however be significantly enhanced by addition of ion-pairing agent such as anionic surfactant. On contrary, the co-extraction of the ion pair MB-anionic surfactant can be used for the determination of concentration of anionic surfactants in the samples. The difference in extraction of MB and MB-anionic surfactant ion pair was examined on the model system with DBSA. A 0.2 mM solution of methylene blue (MB) in deionized water was aspirated through the bottom of the channel 1 and hexanol was aspirated through the top. The DBSA solution in the range 0-20 ppm was injected into the aqueous phase and the extracted ion pair of DBSA-MB was measured using a capillary cell UV detector. The calibration for DBSA is depicted on FIG. [[13]] 12a. The calibration was linear in the range 0-20 ppm ( $r^2=0.998$ ). On FIG. [[13]] 12b and [[13]] 12c, the repeatability of 10 injections of 6 and 12 ppm of DBSA, respectively, is shown. The r.s.d. values ranged from 3.3% and 8.6%. On FIG. [[14]] 13, 3 injections of a blank, e.g. deionized water and 3 injections of tap water into MB-stream were performed. The blank trace shows small negative peaks, resulting from dilution of MB carrier stream. The peaks for tap water correspond to MB-extractable matter.

Pages 32/33, the paragraph spanning page 32, lines 29-31 through page 33, lines 1-7:

**Ion-pair extraction of chromate into butanol.** The ion-pair assisted extraction in the system with horizontal flows can be further exploited by extracting an anionic molecule from the aqueous phase with the ion-pairing reagent incorporated in the organic phase.. The chromate anion is not extracted into butanol, however addition of 2% TOABr into the butanolic phase allowed the transfer of  $\text{CrO}_4^{2-}$  into the organic phase with Br being released into the aqueous phase. A 10 mM NaOH was used as a carrier solution and aqueous solution of potassium chromate was injected into this stream. The repeatability of 12 injections of 1 mM  $\text{K}_2\text{CrO}_4$  extracted into the 2% butanolic solution of TOABr is shown on FIG. [[15]] 14, with r.s.d. of 2.6%.

Page 33, first full paragraph (lines 9-24):

**Conductivity suppression.** In the first section, we have demonstrated continuous ion exchange in side-by-side flows in a shallow planar channel. A lipophilic ion exchanger was dissolved in an

immiscible organic phase flowing parallel side-by side with the eluent stream of strong mineral acid (1-10 mM HCl). A strongly basic compound, such as tetraoctylammonium hydroxide (TOAOH), or a secondary amine, e. g., Amberlite LA-2, were used and proved to be equally efficient for suppression of the conductivity of the eluent phase. Amberlite LA-2 was used exclusively in this study, since it does not significantly change the interfacial surface tension of butanol/water, as does the addition of TOAOH. FIG. [[16a]] 15A shows 12 injections of 1 mM NaCl into a 1 mM HCl eluent, using 1% Amberlite LA-2 in butanol as the ion exchanger. The flow rates of the eluent and ion exchanger were  $20 \mu\text{L min}^{-1}$ . The calibration was linear in the range 0-2 mM NaCl (see FIG. [[16b]] 15B) and the estimated S/N=3 limit of detection (LOD) from these data is  $\leq 0.1$  mM NaCl, largely controlled by dispersion in the injector and the suppression device as well as the larger than optimum detection electrodes; the detector electronics used here is also dated.

Page 34, paragraph 3 (lines 8-18):

Device. Whitesides and Stroock<sup>25</sup> have recently described versatile flexible methods to construct microfluidic devices, especially by soft lithography. We have recently described a “strings and sealing wax” approach to experimenting with flow in shallow planar devices using tapes/thin sheets as spacers and with binder clips to hold things together. The present device constituted of a 6 mm thick 25 x 75 mm fluorinated ethylene-propylene copolymer (FEP) sheet. A channel (50 x 1 x 0.1 mm L x W x D, computed volume 5  $\mu\text{L}$ ) with a trifurcation at each end (constituting 3 inlets and 3 outlets with 0.5 mm apertures drilled through the FEP sheet) was inscribed on the FEP surface by conventional machining. The channel was sealed at the top by a microscope slide, held together by binder clips.—~~The device is shown in Figure 1a.~~

Pages 34/35, the paragraph spanning page 34, lines 30-31 through page 35, lines 1-12:

Initial experiments also showed that the hydraulic resistance of the device is relatively small. Even a 10 cm hydrostatic head produced flow rates much higher than that desired. The respective liquids were therefore aspirated by a peristaltic pump (Miniplus 2, Gilson) from the device outlets at 2-10  $\mu\text{L}/\text{min}$ . The liquids were aspirated at given flow rates from all three outlets, thus the total volumetric flow rate was the sum of the individual flow rates from the three outlets. The inlet liquid reservoirs were connected to the device with large bore tubing (1.25 mm) to eliminate any flow resistance. Normally the inlet reservoirs were maintained at a hydrostatic height of 10 cm. However, the reservoir heights were adjusted to modify the widths of the individual streams flowing through the channel, from only one

liquid, to width ratios of 1:3 to 3:1 (see panels a-c in Figure 1). The change in width effectively changes the residence time. For quantitative exchange experiments, it also changes the mean diffusion distance for the exchangeable ion in the “eluent” stream to the exchange interface.

Page 37, last paragraph (lines 7-19) (following “**Example 2**”):

Figure [[4]] 3a shows the effect of different flow rates in the experimental system of Figure 1 Example 1. Each phase was 500  $\mu\text{m}$  wide. 10 mM HCl and 150 mM butanolic TOAOH were used as eluent and exchanger phases, respectively. In general, the results conform well with the computational results shown in Figure [[3]] 2; however, small leaching of the ion exchanger into the aqueous phase makes it more difficult to exactly compute the fraction exchanged as the fraction exchanged value gets higher. As predicted, the fraction exchanged increases with decreasing flow rates. The contact time in the present device with phase widths of 500  $\mu\text{m}$  and flow rates of 2  $\mu\text{L}/\text{min}$  is  $\sim$ 50 s. As predicted in Figure [[3a]] 2A, a greater degree of suppression can be achieved by decreasing the width of the eluent phase. In the present experimental system, this is most easily achieved by adjustment of relative solution reservoir height s. Figure [[4]] 3b shows the effect of decreasing the width of the eluent phase from 750  $\mu\text{m}$  to 500  $\mu\text{m}$  to 250  $\mu\text{m}$ .

Page 38, first paragraph (following “**Example 3**” (lines 3-12):

Figure [[5]] 4 shows the background conductance achieved with 10 mM HCl and 10 mM NaOH eluents, using an eluent flow rate of 2  $\mu\text{L}/\text{min}$  and an eluent phase width of 250  $\mu\text{m}$ . The suppressed background conductance attained with TOAOH used in conjunction with an acid eluent is much lower than that obtained with an NaOH eluent and various alkylsulfonic acid ion exchangers. The leaching of the acids into the aqueous phase (Table 1) appears to be the main limitation. (We believe that this problem can be solved by using lipophilic alkali metal complexing agents; preliminary experiments with sym-(*n*-decyl)dibenzo16-crown-6-oxyacetic acid, for example, showed promising results.)

Page 38, second paragraph (following “**Example 4**” (lines 16-21):

Figure [[6]] 5 shows triplicate injections of 0.5 mM NaCl into a 1 mM HCl eluent, using both 15 mM TOAOH or 1% Amberlite LA-2 as the ion exchanger. The estimated S/N=3 limit of detection (LOD) from these data is  $\leq$  0.1 mM NaCl, largely controlled by dispersion in the injector and the suppression device as well as the larger than optimum detection electrodes; the detector electronics used

here are also dated.

Page 38, third paragraph (following "Example 5") (lines 25-31):

Figure [[7]] 6 shows conductivity suppression experiments with colloidal aqueous ion exchanger suspension. The efficiency is similar for anion and cation exchanger suspensions and, as such, may be predicted from an effect comparable to that observed for the TOAOH system. The residual conductance probably arises from impurities in the ion exchanger suspension generated during grinding, etc., since the suspension was not purified. Various concentrations of NaCl were injected from a sub- mM to 10 mM level in a 10 mM NaOH –Dowex 50WX8 suspension system. The high concentrations resulted in peaks exceeding 100  $\mu$ S/cm in height. Similar results were achieved for the suppression of 10 mM NaOH with the anion exchanger resin suspension. One obvious benefit of using resin suspensions is that no organic solvents are involved.

Page 39, first full paragraph (following "Example 6") (lines 8-14):

Figure [[8]] 7 demonstrates the feasibility of conductometric detection of a range of heavy metals injected into the system with Amberlite LA-2. Note that even metals such as silver or copper can be detected, though their sensitivity is somewhat lower than the corresponding sensitivity for other metals, probably due to their complex formation with the secondary amine present in the exchanger phase. This approach can potentially have an appropriately functionalized membrane-based analog in a macrosystem.